

The Mechanical Properties of Single Chromatin Fibers Under Tension

S. H. Leuba¹⁾, J. Zlatanova²⁾, M. A. Karymov^{1, 6)}, R. Bash³⁾, Y-Z. Liu⁴⁾, D. Lohr³⁾, R. E. Harrington⁵⁾, and S. M. Lindsay⁴⁾,

¹⁾ Physical Molecular Biology, LRBGE, National Cancer Institute, NIH, Bethesda, MD 20892, USA

²⁾ Argonne National Laboratory, Argonne, IL 60439, USA

³⁾ Department of Chemistry and Biochemistry, ⁴⁾ Department of Physics and Astronomy and ⁵⁾ Department of Microbiology, Arizona State University, Tempe, AZ 85287, USA

⁶⁾ On leave from: Research Institute of Physics, St. Petersburg State University, 198 904 St. Petersburg, Russia

Correspondence to:

Dr. Stuart Lindsay Department of Physics and Astronomy
Arizona State University
Tempe, AZ 85287, USA
Phone 480-965-4691
Fax 480-965-7954
E-mail Stuart.Lindsay@asu.edu
leuba@nih.gov

submitted 10 Jul 2000

published 17 Jul 2000

Abstract

An atomic force microscope was used to image and stretch single synthetic chromatin fibers consisting of twelve core nucleosomes with no linker histones. Peaks in the force-curves are attributed to sequential detachment of nucleosomes from the glass support. The short distances between peaks and reversibility of the pulling process show that the nucleosomes remain intact even at tensions on the order of 350 picoNewtons (pN). This is more than an order of magnitude larger than the force required to de-spool histone octamers from the nucleosomal DNA in laser optical

tweezer measurements made with longer molecules, suggesting that loading rates and the length of the molecule are important factors in determining the force required to break inter-molecular bonds.

Introduction

The nucleosomal packaging of eukaryotic genes would appear to present a major barrier to RNA polymerase during transcription, and the mechanism for dealing with nucleosomal templates has been the subject of much debate [1, 2, 3, 4, 5 and 6]. The tension at which nucleosomes might be released is important for understanding chromatin function. Simple energetic calculations [7] suggest that a tension of a few pN should release the octamer core from DNA. Such small tensions are easily generated by molecular motors [8], yet it appears that transcription through the nucleosome [6] may be a much more complex process than a simple tension-driven release of histones.

The atomic force microscope (AFM) and laser optical tweezers (LOT) are two methods currently used for determining the strength of inter- and intra-molecular bonds. The atomic force microscope can pull on individual molecules, yielding force versus extension curves [9, 10, 11, 12, 13 and 14]. Longer molecules, attached to micron-sized beads, can be stretched by LOT to yield similar data [15, 16, 17, 18]. In both experimental approaches force is generated by extending the molecule (at rates from nm to μm per second) against the restoring force of an AFM cantilever (spring constant ~ 1 N/m) or an optical trap (spring constant $\sim 10^{-5}$ N/m). Force increases with molecular extension according to Hooke's law ($F = -k\Delta x$ where k is the spring constant and Δx is the extension). The probability of bond rupture upon stretching increases both with loading time and with the applied force [19]. The important parameter in these experiments is the loading

rate, the rate at which force is increased per unit time, dF/dt . In simple cases, the bond strength is simply proportional to the logarithm of the loading rate, as normalized to a molecular thermal loading rate (see below). The molecular parameters that determine this molecular loading rate are not known *a priori*, so it is important to determine bond-strengths over as large a range of loading rates as possible. The loading rate in our AFM experiments is on the order of tens of thousands of pN/s while for LOT, it is typically a few pN/s.

The LOT and AFM experiments also differ in the size of the stretched molecule or molecular complex, and this, in turn, modifies the internal loading rate [20]. Long structures generate internal forces more slowly at a given external loading rate than shorter structures, enhancing the probability of bond-rupture at a given external loading rate in the longer molecule, further amplifying the difference between the two experimental approaches [20]. Applying this same argument to chromatin fibers would suggest that the apparent loading rate that a single nucleosome in a long chain of nucleosomes experiences is much smaller than the apparent loading rate experienced by a single nucleosome in a short nucleosomal array under the same given external loading rate.

Recent LOT experiments using micron-long chicken erythrocyte chromatin fibers [21] or chromatin fibers reconstituted from λ -DNA and core histones by a frog egg extract [22] show evidence of non-reversible changes that can be accounted for by the unwinding of DNA from the nucleosomes at forces of a few tens of pN. Although the piconewton force-scale of these experiments is similar to the force measured in molecular motors, it is not probable that forces are applied to micron-sized lengths of DNA *in*

vivo. It is therefore of interest to examine shorter chromatin constructs by AFM.

The AFM also offers the possibility of imaging the molecules that are pulled, greatly facilitating the interpretation of the force data [23]. We have used the imaging capability of the AFM to image small, well-defined chromatin constructs prior to pulling on them to obtain force data. We have also exploited an oscillating-cantilever technique in order to separate reversible and non-reversible features in the pulling curves. With these short samples and the large loading rates of the AFM, we find chromatin to be remarkably strong. Complete de-spooling of the nucleosomal DNA from the histone octamer rarely occurs even at tensions on the order of 350 pN. This is in sharp contrast to LOT experiments on long chromatin fibers. This discrepancy suggests that both loading rates and the length of the molecule under tension are important parameters in controlling the rupture of inter-molecular bonds [20].

Materials and Methods

Preparation of reconstituted 208-12 nucleosomal arrays.

Nucleosomal arrays were reconstituted from chicken erythrocyte core histones [24] and 208-12 DNA [25] via salt dialysis [26]. 5 μ g of 208-12 DNA were mixed with 6 μ g of core histone octamers in 16 μ l 2 M NaCl, 10 mM Tris/HCl, pH 8.0, 0.1 mM EDTA, and then dialyzed sequentially at 4°C for 3 h each versus 10 mM Tris/HCl, pH 8.0, 0.1 mM EDTA

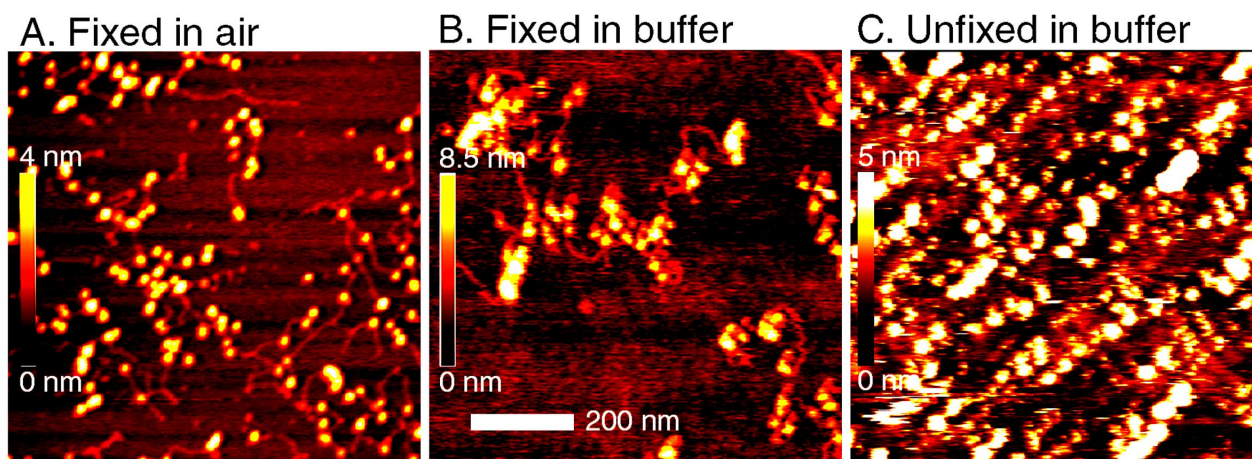


Fig. 1. MacMode AFM images of (A) 208-12 sea urchin DNA (see text) reconstituted with core histones and fixed with 0.1% glutaraldehyde, imaged in air on glass, (B) the same material imaged in 5 mM triethanolamine/HCl, pH 7.0, 0.1 mM EDTA and (C) unfixed material in the same buffer. The orientation of the unfixed molecules on the surface appears after a rinsing step. The scan sizes are 800 nm by 800 nm. Heights are coded by color with low regions depicted in dark brown and higher regions in increasingly lighter shades, as indicated in the vertical scales (bars on left) in each image.

containing 1 M NaCl or 0.75 M NaCl, and finally versus 5 mM triethanolamine/HCl, pH 7.0, 0.1 mM EDTA. Alternatively, the DNA/histone mixture in 2 M NaCl was reconstituted via a salt-jump method [27] by incubation at 37°C for 15 min, dilution with three volumes of 10 mM Tris/HCl, pH 8.0, 0.1 mM EDTA at the same temperature, further incubation at 37°C for 15 min, and then dialysis for 3 h at 4°C versus 5 mM triethanolamine/HCl, pH 7.0, 0.1 mM EDTA.

Glutaraldehyde Fixation of Nucleosomal Arrays.

For some experiments, reconstituted nucleosomal arrays ($A_{260} = 2$) in 5 mM triethanolamine/HCl, pH 7.0, 0.1 mM EDTA were fixed in 0.1 % electron microscopy grade glutaraldehyde overnight before deposition for AFM imaging/manipulation [28].

Atomic Force Microscopy.

Nucleosomal arrays ($A_{260} = 2$) in 5 mM triethanolamine/HCl, pH 7.0, 0.1 mM EDTA were deposited for 2 min onto a hydrogen-flame annealed glass coverslip, rinsed, and imaged in the same buffer. Images were obtained with a MacMode dynamic force microscope (Molecular Imaging, Phoenix, AZ) operated with MacLevers of force constant 0.5 ± 0.1 N/m, at an oscillation frequency of 25 kHz, and a 0.2 nm reduction in the 2-3 nm free-amplitude vibration of the cantilever. For acquiring force curves, the tip was typically advanced towards the surface a distance of 400 nm, with an initial force applied into the surface of more than 200 nN, and subsequently retracted. Pulling speeds were in the range of 72-2000 nm/s. Little dependence of the force curves on loading rates was found over the corresponding range of 3.6×10^4 pN/s to 1×10^6 pN/s. The uncertainty of the force constant of the cantilevers is ~20%, and tip deflections and distances were calibrated to better than 5% leading to uncertainties in the measured force of about 25%. The error owing to the tip deflection contribution to the overall extension values is small.

Forces and Oscillation Amplitude Curves.

Forces and oscillation amplitudes were recorded simultaneously as described by Liu et al. [29]. The comparison of the force curves with the integrated amplitude curves serves as a test of the local reversibility of the pulling process. For a purely elastic response, the stiffness of the molecule at some point in the pull (distance z) is calculated from

$$S(z) = -k \left(\frac{A_0}{A(z)} - 1 \right)$$

where A_0 is the undamped amplitude (typically 2 nm here), $A(z)$ is the amplitude at a distance z , and k the spring

constant of the cantilever. If the mechanical response is purely elastic, the force is obtained by integration,

$$F(z) = -k \int \left(\frac{A_0}{A(z)} - 1 \right) dz + C$$

Results

Initial AFM studies of chicken erythrocyte chromatin yielded force curves with an irregular series of peaks [30], reminiscent of the more regular sawtooth-like force curves obtained by pulling on titin [9]. Such curves were not observed in control experiments with DNA alone, and clearly reflect chromatin structure in some way. In order to clarify their origin, we turned to a model system, a nucleosomal array, which consists of 12 tandem repeats of a 208 bp sequence from the 5S rRNA gene of the sea urchin *Lytechinus variegatus*, each containing a single positioned nucleosome. Each 208 bp sequence positions a nucleosome at one of several closely-spaced discrete locations [31, 32], producing a relatively regular nucleosome array.

The loading of core histones on the 208-12 DNA in the model fibers was confirmed by AFM images of fixed samples obtained in air (Figure 1A) [33]. Atomic force microscope images obtained in buffer are shown in Figure 1B for fixed chromatin and Figure 1C for unfixed chromatin. Figures 1A and 1B demonstrate that the array of nucleosomes in the fixed chromatin fibers is much more condensed than the same material imaged in air, presumably because it has not been affected by drying. The unfixed chromatin in buffer appears as short linear arrays of nucleosomes; only a few of the unfixed fibers have more than two (presumably) connected nucleosomes. It appears that when deposited and imaged in solution the unfixed material did not attach to the substrate along its entire length.

When the tip was placed over a molecule, driven into it and subsequently retracted, force-distance and amplitude-distance curves showed periodic features such as those illustrated in Figures 2A and 2B (data for unfixed molecules). The analysis was restricted to experiments in which clear AFM images of well-isolated molecules were obtained prior to pulling, because this was an essential requirement for obtaining simple, reproducible force curves. Several remarkable features are immediately apparent from these curves. The height of the force peaks is on the order of ~350 pN, two orders of magnitude greater than the tension required to release bound histone octamers according to theoretical thermodynamic arguments [7]. When a given molecule is pulled repeatedly, those peaks that reappear are located at reproducible positions (Figure 2B), an observation that cannot be reconciled with an irreversible process such as removal of the histone octamers from the DNA.

Comparison of the force- and oscillation-amplitude curves is also interesting. Figure 2C shows that the curves leading up to each peak are well-fitted as a simple elastic process, using the integration described in *Materials and Methods* [29]. The shape of the curves is similar to that obtained when much shorter DNA is stretched to the S form [34]. Another clue to the nature of the stretching process is provided by the relative strength of successive force peaks (F_1 , F_2 , F_3 , in Figure 2A). If the first and last nucleosomes on a fiber were attached to opposing solid surfaces with the rest of the fiber completely suspended in solution and under tension, the weakest nucleosome in the suspended portion of the fiber should be disrupted first, resulting in the ordered series of peaks as seen in titin denaturation experiments [9]. In general, we see no relationship between the maximum force of a peak and the order of its appearance. Many curves (e.g. Figure 2A) show just the opposite behavior with the strongest peak appearing first, implying that the whole fiber cannot be under tension at the start of the pull.

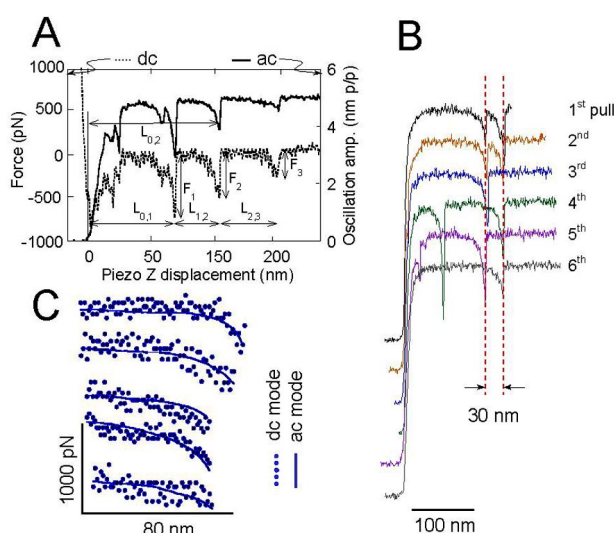


Fig. 2. (A) Simultaneously-obtained conventional force curve (dc mode signal – dashed line) and amplitude oscillation curve obtained with an applied modulation (ac mode signal – solid line) from a 208-12 nucleosomal array as it was pulled off a glass substrate in 5 mM triethanol-amine/HCl, pH 7.0, 0.1 mM EDTA. F_1 , F_2 , and F_3 (F_n) indicate three force peaks. $L_{n,n+1}$ indicate peak-to-peak distances, $L_{0,n}$ indicate distances from the origin of the pull to the n^{th} peak. (B) Sequential ac retraction curves for repeated pulling on the same molecule. The two vertical dashed red lines are centered on two peaks that reappear in repeated pulls. (C) Comparison of force calculated from measured amplitude oscillation curves (ac mode signal – lines) with measured force (dc mode signal – dots) as described in the *Materials and Methods*. Similar agreement was found for 22 curves selected at random, indicating that the process leading up to each peak is elastic.

The images (Figure 1C) suggest an explanation consistent with these observations, which is that the peaks correspond to the removal of successive *intact* nucleosomes from the glass substrate, as illustrated in Figure 4. After the first nucleosome is pulled away by the tip, the DNA stretches by a factor between 1.5 and 2 times at these forces [34, 35] until the next nucleosome pops off the substrate, relaxing the DNA. The DNA is then stretched again (to a total length L) until the process repeats. This ‘popping-off-intact’ model leads to much shorter distances between successive force peaks, because the DNA remains wound on the nucleosome.

Statistical summaries of the pulling data for the unfixed molecules are shown in Figure 3. Figure 3A shows the frequency distribution of the number of peaks per force curve alongside the frequency of a given number of nucleosomes per fiber in the AFM images. Only a fraction of each unfixed fiber attaches to the substrate, the most common number of contiguous visible nucleosomes being just two. The ‘popping-off-intact’ model of the force curves predicts $N-1$ peaks for a fiber composed of N nucleosomes, exactly as observed (Figure 3A). The distances between peaks ($L_{n,n+1}$ in Figure 2A) are summarized in Figure 3C. A Gaussian fit to the observed distances has a maximum at ~ 30 nm along with a tail of higher values. The center-to-center internucleosomal distances measured from the images (Figure 1c) is 39 nm (x in Figure 4), and taking the diameter of a nucleosome to be 11 nm, we arrive at a calculated vertical displacement between peaks ($L_{n,n+1}$ in Figure 2A) in the range of 36 to 55 nm (corresponding to S-DNA extensions of between 1.5 and 2 times). If the DNA were to de-spool completely from the histone octamers, much larger distances would be found (see Table 1).

Table 1. Comparison of modeled and observed peak-to-peak distances.

	ΔZ – Nucleosomes de-spooled (nm)	ΔZ – Nucleosomes remain intact (nm)	ΔZ Observed (nm)
Un-fixed	105 to 142	36 to 55	48±40 (N=49)
Fixed	459 to 619	104 to 155	120±100 (N=428)

The peak forces are bimodally distributed as shown in Figure 3B with peaks at 325 ± 10 and 550 ± 30 pN. In our ‘popping-off-intact’ model, such a distribution is expected. One peak comes from processes that pick up a fiber at one end only, and the other from processes that pick up a fiber internally (for which the forces are doubled, see the inset in Figure 3B). The measured ratio of the number of ‘internal’ to the number of ‘end’ processes, as determined from the

relative areas of the two Gaussians used to fit the data (shown in Figure 3B) is 0.42 ± 0.02 . This is roughly consistent with calculations based on the distribution in Figure 3A which yield a calculated value of 0.15.

The distribution of distances from the origin of the pull to each peak are also completely inconsistent with total removal of the histone octamers. A fully extended S-DNA of 11 tandem repeated DNA linkers (with the nucleosomes intact) would be about 700 nm in extended fiber length. Values for $L_{o,n}$ (Figure 2A) are summarized in Figure 3D. The largest observed value is less than 250 nm, again a lot less than what would be expected if the nucleosomes de-spooled.

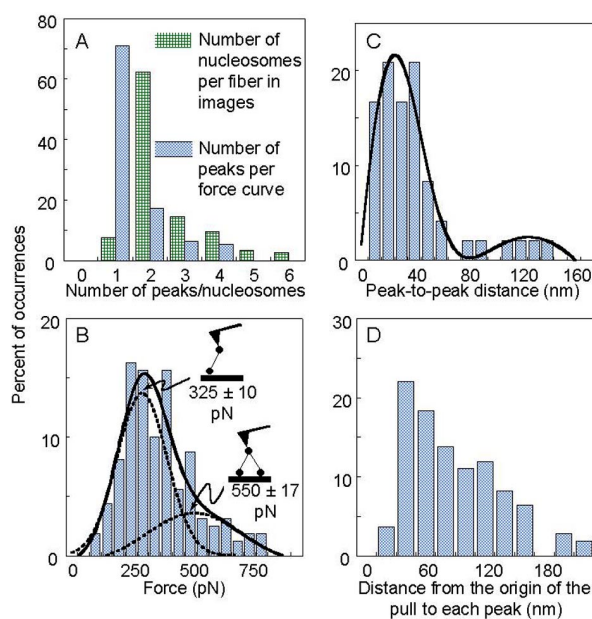


Fig. 3. Statistical summary of data for the unfixed molecules: **(A)** Histogram of the number of peaks per force curve (diagonal crosshatch) juxtaposed with the number of nucleosomes per array from the AFM images (horizontal crosshatch). **(B)** Histograms for the peak forces, **(C)** Peak-to-peak distances, and **(D)** Distances from the origin of the pull to each peak in the force curves. The solid lines in **(B)** and **(C)** represent best fits to a two-Gaussian distribution. The insets in **(B)** illustrate the model for the two force peaks.

If the histone octamers are not removed from the fibers, fixing them should have little effect on the observed force peaks. We studied fibers that were fixed with 0.1% glutaraldehyde. These adopted a different conformation on the substrate (Figure 1B) but the peak forces were similar, with a bimodal distribution peaking at 340 ± 10 pN and 580 ± 50 pN. The peak-to-peak-distance distributions and ratio of 'internal' to 'end' processes were different, but these differences were quantitatively accounted for by the 'popping-off-intact' model, given the different conformation on the substrate. Images of the fixed material show an

average of 9.6 ± 2.4 ($N=43$) visible nucleosomes per fiber. More DNA is wound onto each histone octamer, the center-to-center distance between adjacent nucleosomes being 28 ± 8 nm ($N=837$) compared to 39 ± 6 nm ($N=175$) for the unfixed material. However, fewer nucleosomes contacted the surface, as evident from AFM images and the pulling curves: there were only 2 ± 1 peaks on the average in the force curves ($N=419$), yielding an average of 4.4 nucleosomes between attachment points. Thus, the horizontal distance between attachment points is 4.4×28 nm, leading to the calculated peak-to-peak distances listed in Table 1.

Discussion

Laser-tweezer studies stretching chicken erythrocyte chromatin fibers containing all the histones show evidence of irreversible changes when the applied force exceeds about 20 pN (though it appears that the DNA is not completely stripped of all proteins at tensions of 65 pN on the first pull) [21]. Pulling experiments on chromatin assembled directly in the liquid cell onto a λ -DNA with *Xenopus laevis* egg extract show a clear sawtooth pattern in the force extension curve with 20 pN peaks in which the fiber length increases by the amount expected for DNA de-spooling from the nucleosome [22].

The present AFM experiments differ from these LOT measurements in several significant ways. The chromatin fibers used by Cui and Bustamante contain linker histone H1 and H5, in addition to core histones, and it is well known that the presence of linker histone adds levels of structural complexity to a chromatin fiber [36, 37]. The fibers prepared by Bennink *et al.* are more comparable to the artificial chromatin construct used here because they lack somatic linker histones and are of a defined length; however, the fibers in Bennink *et al.* were prepared in the presence of a cell extract, protein components of which may associate with the fiber in contrast to the chromatin fibers used here which contain core histones only. The chromatin fibers used in both of these LOT experiments were long (on the order of hundreds of nucleosomes). Our templates are small (12 nucleosomes) and relatively homogenous in both size and composition. Finally, our extension rates are substantially higher. The LOT experiments of Bennink *et al.* [22] were carried out at rather small loading rates of $dF/dt = 100\text{--}150$ pN/s. The measurements reported here were carried out at rates between 35×10^3 and 10^6 pN/s, with little evidence of a rate dependence in the force curves. The process we believe we are observing (popping off of the nucleosomes off the glass surface) probably has a completely different loading rate dependence from the process observed in the LOT experiments (de-spooling of nucleosomes).

The AFM data set a *lower* limit of about 350 pN for the de-spooling process for these short fibers pulled at these higher rates. Thus, an increase in loading rate of about

350 times (using our smallest loading rate and the LOT rate of 100 pN/s) results in an increase in strength of at least ~20 times. This is a much larger effect than observed in the mechanical unfolding of titin domains where a similar loading rate increase only strengthens the domains by a little over a factor 2 [10, 20].

Quantitative comparison of the two techniques is difficult to carry out because the scaling of loading rates depends upon the parameter f_β/t_0 . f_β is a thermal bond strength, given by $k_B T/x_\beta$ where x_β is a characteristic bond-length, and t_0 is the bond lifetime at zero force [20]. In terms of this parameter, the bond strength is given approximately by $f_\infty \ln [(dF/dt)/(f_\beta/t_0)]$ if the internal molecular compliance is negligible. A twenty fold increase in bond strength between the LOT and AFM would require $f_\beta/t_0 = 73$ pN/s, more than thirty thousand times greater than observed for titin [20]. This large value is consistent with our AFM data in as much as the change in bond strength over the entire range of loading rates we have explored is only about 40%, and our measurements serve only to set a lower limit.

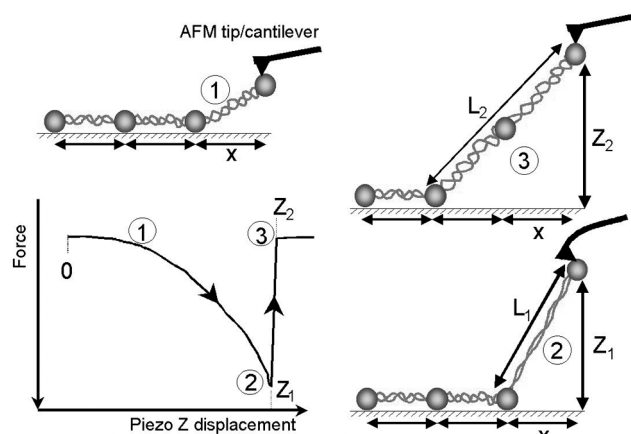


Fig. 4. Schematic of stretching a nucleosomal array illustrating the geometry for calculating the peak-to-peak distances in the 'popping-off' model (see text), and its associated AFM force curve. The AFM tip lifts one end of the fiber off the surface (1); stretching of linker DNA between the tip and surface, leading to an increase in force (2); stretched linker DNA relaxes by the adjacent nucleosome popping off the surface without de-spooling, leading to relaxation of the stress on the cantilever (3). X is the horizontal distance between centers of adjacent nucleosomes; Z is the vertical distance from the tip to the surface; and L is the distance from the tip to the first nucleosome on the surface.

The strongest sample-dependent variation in f_β/t_0 arises from the exponential dependence of t_0 on bond strength, and the data would require about a ten-fold increase in the number and/or strength of bonds between those stabilizing the β -barrel in a titin IgG-like domain and those participating

in the maintenance of the nucleosome structure. Another important difference comes from the overall size of the samples used in the two experiments (about an order of magnitude in DNA length). Discrimination between these two contributions would be possible if more data on the loading-rate dependence of the de-spooling force were available [20]. Nonetheless, comparison of the two types of experiment suggests a remarkably strong dependence of the de-spooling force on loading rate. This, in turn, suggests that kinetic effects are extremely important in determining the consequences of the forces applied to nucleosomes by motor proteins.

Thus, we conclude that the main process generating the elastic portion of the AFM force-distance curves is reversible stretching of the linker DNA (Figure 4). Some partial unwrapping of the DNA from around the histone octamer cannot be excluded as expected both from the extended fiber geometry [38, 39] and the weaker contacts near the ends of the nucleosomal DNA [40; 41, 39], but it must contribute relatively little to the AFM force curves. The 'collapse' part of the curves (Figure 4) evidently reflect pulling away of the nucleosomes from the glass substrate rather than removal of the histone octamer from the nucleosome core. We note that the adhesion energy obtained from integrating these peaks ($3.5 \pm 3 \times 10^{-18}$ J) is reasonable for a molecule-substrate contact area on the order of 100 nm^2 [42].

Finally, we turn to consider the factors that might stabilize the core particle in these experiments. Removal of the core particle requires torsional freedom in the assembly. This is clearly very limited at the start of the pull when one nucleosome is attached to the tip and the other attached to the surface. When one nucleosome is suspended between the one attached to the tip, and the other attached to the surface, approximately one turn of superhelical winding ($\Delta\phi = 2\pi$) must be released into about 100 nm of total DNA length. Using a torsional persistence length of 100 nm [43] leads to a torsional energy of about 10^{-21} J, three thousand times less than the elastic energy stored in 100 nm of DNA at 300 pN tension. It seems likely, therefore, that the nucleosome must distort in some way to trap the DNA. Following Cui and Bustamante (2000), we estimate the pressure on the nucleosome core to be about 3×10^7 Pa in the AFM experiments. This is still on the order of 100 times less than the compressional modulus of a typical protein. Thus any 'pinching' of the protein core must be small.

Whether chromatin is durable or fragile under tension clearly depends upon the rate at which it is loaded and the length over which the tension is applied. The values of these parameters in processes in eukaryotic cells is unknown at present.

Acknowledgements: We thank Carlos Bustamante for providing us with a copy of their manuscript prior to

publication, and M. L. Bennink for useful discussions. This research was supported by grants from NSF (BIR 9513233), NIH (CA70274), and Molecular Imaging Corporation (TCL-157C) to S.M.L. S.H.L. is an NCI Scholar.

References

- [1] Losa, R. and Brown, D.D. (1987) A bacteriophage RNA polymerase transcribes in vitro through a nucleosome core without displacing it. *Cell*, **50**, 801-808.
- [2] Lorch, Y., LaPointe, J.W. and Kornberg, R.D. (1988) On the displacement of histones from DNA by transcription. *Cell*, **55**, 743-744.
- [3] van Holde, K.E., Lohr, D.E. and Robert, C. (1992) What happens to nucleosomes during transcription? *J. Biol. Chem.*, **267**, 2837-2840.
- [4] Studitsky, V.M., Clark, D.J. and Felsenfeld, G. (1994) A histone octamer can step around a transcribing polymerase without leaving the template. *Cell*, **76**, 371-382.
- [5] Studitsky, V.M., Clark, D.J. and Felsenfeld, G. (1995) Overcoming a nucleosomal barrier to transcription. *Cell*, **83**, 19-27.
- [6] Bednar, J., Studitsky, V.M., Grigoryev, S.A., Felsenfeld, G. and Woodcock, C.L. (1999) The nature of the nucleosomal barrier to transcription: direct observation of paused intermediates by electron cryomicroscopy. *Mol. Cell*, **4**, 377-386.
- [7] Marko, J.F. and Siggia, E.D. (1997) Driving proteins off DNA using applied tension. *Biophys. J.*, **73**, 2173-2178.
- [8] Wang, M.D., Schnitzer, M.J., Yin, H., Landick, R., Gelles, J. and Block, S.M. (1998) Force and velocity measured for single molecules of RNA polymerase. *Science*, **282**, 902-907.
- [9] Rief, M., Gautel, M., Oesterhelt, F., Fernandez, J.M. and Gaub, H.E. (1997) Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science*, **276**, 1109-1112.
- [10] Rief, M., Oesterhelt, F., Heymann, B. and Gaub, H.E. (1997) Single Molecule Force Spectroscopy on Polysaccharides by Atomic Force Microscopy. *Science*, **275**, 1295-1297.
- [11] Oberhauser, A.F., Marszalek, P.E., Erickson, H.P. and Fernandez, J.M. (1998) The molecular elasticity of the extracellular matrix protein tenascin. *Nature*, **393**, 181-185.
- [12] Carrion-Vazquez, M., Oberhauser, A.F., Fowler, S.B., Marszalek, P.E., Broedel, S.E., Clarke, J. and Fernandez, J.M. (1999) Mechanical and chemical unfolding of a single protein: a comparison. *Proc. Natl. Acad. Sci. USA*, **96**, 3694-3699.
- [13] Marszalek, P.E., Lu, H., Li, H., Carrion-Vazquez, M., Oberhauser, A.F., Schulten, K. and Fernandez, J.M. (1999) Mechanical unfolding intermediates in titin modules. *Nature*, **402**, 100-103.
- [14] Marszalek, P.E., Oberhauser, A.F., Pang, Y.P. and Fernandez, J.M. (1998) Polysaccharide elasticity governed by chair-boat transitions of the glucopyranose ring. *Nature*, **396**, 661-664.
- [15] Smith, S.B., Cui, Y. and Bustamante, C. (1996) Overstretching B-DNA: the elastic response of individual double-stranded and single-stranded DNA molecules. *Science*, **271**, 795-799.
- [16] Kellermayer, M.S., Smith, S.B., Granzier, H.L. and Bustamante, C. (1997) Folding-unfolding transitions in single titin molecules characterized with laser tweezers. *Science*, **276**, 1112-1116.
- [17] Hegner, M., Smith, S.B. and Bustamante, C. (1999) Polymerization and mechanical properties of single RecA-DNA filaments. *Proc. Natl. Acad. Sci. USA*, **96**, 10109-10114.
- [18] Bennink, M.L., Scharer, O.D., Kanaar, R., Sakata-Sogawa, K., Schins, J.M., Kanger, J.S., de Groot, B.G. and Greve, J. (1999) Single-molecule manipulation of double-stranded DNA using optical tweezers: interaction studies of DNA with RecA and YOYO-1. *Cytometry*, **36**, 200-208.
- [19] Evans, E. and Ritchie, K. (1997) Dynamic strength of molecular adhesion bonds. *Biophys. J.*, **72**, 1541-1555.
- [20] Evans, E. and Ritchie, K. (1999) Strength of a weak bond connecting flexible polymer chains. *Biophys. J.*, **76**, 2439-2447.
- [21] Cui, Y.J. and Bustamante, C. (2000) Pulling a single chromatin fiber reveals the forces that maintain its higher-order structure. *Proc. Natl. Acad. Sci. USA*, **97**, 127-132.
- [22] Bennink, M.L., Leuba, S.H., Leno, G.H., Zlatanova, J., de Groot, B.G. and Greve, J. (2000) Unfolding individual nucleosomes upon stretching single chromatin fibers using optical tweezers. Submitted.
- [23] Müller, D.J., Baumeister, W. and Engel, A. (1999) Controlled unzipping of a bacterial surface layer with atomic force microscopy. *Proc. Natl. Acad. Sci. USA*, **96**, 13170-13174.
- [24] Simon, R.H. and Felsenfeld, G. (1979) A new procedure for purifying histone pairs H2A + H2B and H3 + H4 from chromatin using hydroxylapatite. *Nucleic Acids Res.*, **6**, 689-696.
- [25] Simpson, R.T., Thoma, F. and Brubaker, J.M. (1985) Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure. *Cell*, **42**, 799-808.
- [26] Tatchell, K. and van Holde, K.E. (1977) Reconstitution of chromatin core particles. *Biochemistry*, **16**, 5295-5303.
- [27] Zivanovic, Y., Duband-Goulet, I., Schultz, P., Stofer, E., Oudet, P. and Prunell, A. (1990) Chromatin

- reconstitution on small DNA rings. III. Histone H5 dependence of DNA supercoiling in the nucleosome. *J. Mol. Biol.*, **214**, 479-495.
- [28] Leuba, S.H., Yang, G., Robert, C., Samori, B., van Holde, K., Zlatanova, J. and Bustamante, C. (1994) Three-dimensional structure of extended chromatin fibers as revealed by tapping-mode scanning force microscopy. *Proc. Natl. Acad. Sci. USA*, **91**, 11621-11625.
- [29] Liu, Y.Z., Leuba, S.H. and Lindsay, S.M. (1999) Relationship between stiffness and force in single molecule pulling experiments. *Langmuir*, **15**, 8547-8548.
- [30] Leuba, S.H., Karymov, M.A., Liu, Y.Z., Lindsay, S.M. and Zlatanova, J. (1999) Mechanically stretching single chromatin fibers. *Gene Ther. Mol. Biol.*, Boulikas, T. Ed. **4**, 297-301.
- [31] Dong, F., Hansen, J.C. and van Holde, K.E. (1990) DNA and protein determinants of nucleosome positioning on sea urchin 5S rRNA gene sequences in vitro. *Proc. Natl. Acad. Sci. USA*, **87**, 5724-5728.
- [32] Meersseman, G., Pennings, S. and Bradbury, E.M. (1991) Chromatosome positioning on assembled long chromatin. Linker histones affect nucleosome placement on 5 S rDNA. *J. Mol. Biol.*, **220**, 89-100.
- [33] Allen, M.J., Dong, X.F., O'Neill, T.E., Yau, P., Kowalczykowski, S.C., Gatewood, J., Balhorn, R. and Bradbury, E.M. (1993) Atomic force microscope measurements of nucleosome cores assembled along defined DNA sequences. *Biochemistry*, **32**, 8390-8396.
- [34] Noy, A., Vezenov, D.V., Kayyem, J.F., Meade, T.J. and Lieber, C.M. (1997) Stretching and breaking duplex DNA by chemical force microscopy. *Chem. Biol.*, **4**, 519-527.
- [35] Rief, M., Clausen-Schaumann, H. and Gaub, H.E. (1999) Sequence-dependent mechanics of single DNA molecules. *Nat. Struct. Biol.*, **6**, 346-349.
- [36] Ramakrishnan, V. (1997) Histone H1 and chromatin higher-order structure. *Crit. Rev. Eukaryot. Gene Expr.*, **7**, 215-230.
- [37] Zlatanova, J. and van Holde, K. (1996) The linker histones and chromatin structure: new twists. *Prog. Nucleic Acids Res. Mol. Biol.*, **52**, 217-259.
- [38] Zlatanova, J., Leuba, S.H. and van Holde, K. (1998) Chromatin fiber structure: morphology, molecular determinants, structural transitions. *Biophys. J.*, **74**, 2554-2566.
- [39] Zlatanova, J., Leuba, S.H. and van Holde, K. (1999) Chromatin structure revisited. *Crit. Rev. Eukaryot. Gene Expr.*, **9**, 245-255.
- [40] Luger, K. and Richmond, T.J. (1998) DNA binding within the nucleosome core. *Curr. Opin. Struct. Biol.*, **8**, 33-40.
- [41] van Holde, K. and Zlatanova, J. (1999) The nucleosome core particle: does it have structural and physiologic relevance? *Bioessays*, **21**, 776-780.
- [42] Israelachvili, J.N. (1991) *Intermolecular and Surface Forces*. Academic Press, New York.
- [43] Allemand, J.F., Bensimon, D., Lavery, R. and Croquette, V. (1998) Stretched and overwound DNA forms a Pauling-like structure with exposed bases. *Proc. Natl. Acad. Sci. USA*, **95**, 14152-14157.